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Serum C-reactive protein (CRP) levels and the values of blood hematological indices in piglets*

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The objective of this study was to determine whether variability in serum C-reactive protein (CRP) levels affected values of blood hematological indices in suckling piglets – the offspring of sows differing in serum CRP concentrations. Piglets (n=359) aged 21 ±3 days (younger) and 35 ±3 days (older) were the offspring of Polish Large White x Polish Landrace sows and Duroc x Pietrain boars. Blood samples were collected from the anterior *vena cava* of sows and piglets. The morphological parameters of peripheral blood were determined only in piglets, and serum CRP levels were measured both in piglets and sows. Sows were divided into groups showing low (median = 13 mg/l), average (me = 25 mg/l) and high (me = 36.8 mg/l) serum CRP levels. The values of the analysed parameters in piglets of different origin were compared by the Kruskal-Wallis test and Dunn's test. It was found that offspring of sows with low and medium CRP levels did not differ in terms of blood concentration of this protein. Its concentration was lower than that recorded in blood of piglets from sows with high CRP levels. In younger piglets an elevated CRP level was accompanied by increased monocyte counts and decreased granulocyte counts. It also had an adverse effect on the erythrocyte system, indicating anemia. In older piglets an elevated CRP level, apart from increased monocyte counts, indicated increased thrombocytopoiesis.

KEY WORDS: piglets / sows / C-reactive protein / hematological indices

The first line of defence against pathogens is provided by non-specific immunity, either cellular (e.g. granulocytes, monocytes/macrophages, thrombocytes) or humoral (e.g. the complement system, cytokines, lactoferrin, CRP). The pleiotropically acting C-reactive protein plays both anti-inflammatory and proinflammatory functions [3, 15]. In the body CRP is found in two isoforms exhibiting opposite effects, i.e. the native, pentameric form (CRPp) and the modified, monomeric form (CRPm), which is ascribed strong proinflammatory action [13].

During the acute-phase reaction, i.e. a defence response to inflammation, infection or trauma, serum CRP level in humans or pigs increases rapidly and markedly [3, 15].

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As an acute-phase protein CRP recognises antigens, forms complexes with them and after activation of the complement system participates in their elimination [3, 15]. Moreover, CRP promotes phagocytosis, affecting monocytes, macrophages and neutrophils, as well as exhibits chemotactic and opsonic properties [3, 15]. After the elimination of the pathogen the concentration of CRP returns to the initial level.

Chronically elevated CRP level indicates immune excitation of the organism. The C-reactive protein is not only a marker for this state, but it also participates in its development. This is because CRP induces monocytes/macrophages to synthesise proinflammatory cytokines and inhibits anti-inflammatory cytokine synthesis [2, 22]. Moreover, it enhances the proinflammatory response of interleukin-6 (IL-6) [9, 27] by stimulating its production next to IL-1 and TNF-α (the tumour necrosis factor). In hepatocytes interleukin-6 is the primary inducer of CRP synthesis [3, 15] as well as hepcidin synthesis [8]. Hepcidin is a negative regulator of iron cycle from enterocytes, hepatocytes and macrophages [17]. Its increased blood concentration is associated e.g. with reduction of erythropoietin, thus promoting inhibition of erythroid progenitor cell proliferation [5].

Decreased values of certain blood parameters (erythrocytes, hemoglobin, hematocrit) were recorded in sows with elevated CRP levels [29].

Information on the C-reactive protein in pigs is primarily related with the evaluation of its applicability as a marker of health status. However, practically no data is available on the consequences of individual variation in its concentration. The reference values for this protein level in blood fall within a wide range from 3.6 up to 183 mg/l [6].

The aim of this study was to verify whether variation in the serum CRP concentration plays a role in the modification of hematological markers analysed in a population of suckling piglets differing in age and coming from dams showing low, medium and high levels of this protein.

Material and Methods

The analyses were conducted on suckling piglets (n=359) showing no evident disease symptoms, which dams were crossbred Large White Polish x Polish Landrace sows and sires were crossbred Duroc x Pietrain boars. The experimental piglets were kept in a family farm. The population of piglets was divided into younger animals coming from sows (n=22) being in day 21 (±3) of lactation, and older piglets from sows (n=23) being in day 35 (±3) of lactation. In the first week of life boar piglets were castrated, had their tails docked and teeth clipped and received Suibiofer SE (Biowet, Drwalew SA). Based on the consent (permit no. 9/2008) of the Local Ethics Committee blood for analyses was collected from the anterior vena cava to vials with clotting agent (for CRP analyses) or with EDTA-2 (for hematological tests). Analyses were performed in a veterinary diagnostics laboratory. Serum CRP content was assayed using a latex suspension with human CRP antibodies following the recommendations of Biosystems (Spain) using an EPOLL 20 photometer. Assays of hematological parameters were performed in an NS 4 automatic blood analyser (Cedex). Thus the total leukocyte count was determined, including lymphocytes, monocytes and granulocytes; the erythrocyte count, hemoglobin concentration (Hb), mean corpuscular volume (MCV), hematocrit level (Ht), mean corpuscular hemoglobin (MCH),

mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), large platelet count (LPTL) and platelet distribution width (PDW). Consistency of distribution for levels of these parameters with normal distribution was verified using the Kolmogorov-Smirnov test and the Lilliefors test. Values of these traits were compared between piglets coming from dams having low (me=13 mg/l), medium (me=25 mg/l) and high (me=36.8 mg/l) serum CRP levels.

In view of the lack of consistency in the distribution for values of the traits with normal distribution comparisons were made using the Kruskal-Wallis test with Dunn's test. Traits were presented as medians (me) and their minimum and maximum values were given.

Results and Discussion

A relationship was found between serum CRP concentrations recorded in piglets and respective levels in their dams. Offspring (regardless of their age) coming from sows having high CRP levels showed its higher concentration than it was recorded in blood serum of the other piglets. In this respect offspring of dams with low and medium CRP levels were found not to differ. These results were partly confirmed by earlier population studies [29].

The CRP values in the analysed piglets ranged from 1.1 mg/l to 47.0 mg/l (Table 1), which was a much narrower range than reported by Diack et al. [6]. Marked differences in the concentration of this protein between individual animals were explained by those authors e.g. by the potential effect of polymorphism in the CRP gene or cytokine-encoding genes. This suggestion may be confirmed by the results reported by Łaszyn and Życzko [16] on the CRP gene mutation modifying CRP concentration. It is assumed that the variation in CRP concentration in the piglets may have a genetic background (Table 1).

In the human an approx. 2- to 3-fold greater blood CRP level compared to the physiological value accompanies chronic low-intensity inflammation [18, 25]. During this condition the concentration of proinflammatory cytokines (TNF alfa, IL-1, IL-6) is also elevated. Moreover, changes are observed in the leukocyte system, manifested in an increased count of monocytes, lymphocytes and eosinophils, while the total white blood cell count remained normal [25].

While the count of white blood cells (including lymphocytes) in blood of piglets from dams with either high or low CRP levels did not differ, it was lower than that recorded in younger piglets from dams with a medium CRP level. Offspring of sows with a high CRP level showed a higher count of monocytes in blood in relation to the other piglets (both younger and older) and a lower granulocyte count (only in younger piglets). In this study the median for the leukocyte count fell within the physiological normal range of 11 to 22 x 10^9/l. The reference values for the individual leukocyte fractions are 4.5-13.0 x 10^9/l for lymphocytes, 0.2-2.0 x 10^9/l for monocytes, 3.2-10.0 x 10^9/l for neutrophils, 0.5-2.0 x 10^9/l for eosinophils and 0.0-0.3 x 10^9/l for basophils, respectively [21].

The presented normal limit was exceeded by the monocyte count in blood of piglets with an elevated CRP level. Moreover, minimum and maximum values of analysed parameters differed from the cited ranges. Egeli et al. [7] indicated a particularly high variation

Table 1

Serum CRP level and values of white blood cells' indices in younger (m) and older (s) piglets with consideration of their origin

Indices	Age of piglets	Offspring of mothers with CRP levels: low	medium	high	P value
	m s	n = 109 n = 93	n = 57 n = 36	n = 18 n = 46	
CRP (mg/l)	m	9.8^{a} (1.1–21.1)	7.8^{Aa} (1.6–26.3)	14.1^{Bb} (4.5–27.3)	0.0018
	s	9.2^{a} (1.5–25.0)	8.9^{a} (1.6–25.7)	13.8^{b} (1.2–47.0)	0.0207
White blood cells ($x10^9$/l)	m	14.4^{A} (7.6–35.8)	$16,9^{B}$ (8.4–27.2)	11.9^{A} (6.3–30.6)	0.0003
	s	20.3 (5.2–35.7)	19.9 (4.7–33.1)	19.7 (8.4–39.0)	0.9047
Lymphocytes ($x10^9$/l)	m	6.3^{a} (3.9–19.1)	8.0^{b} (3.2–12.7)	5.8^{a} (3.2–17.2)	0.0301
	s	7.9 (1.1–12.8)	7.8 (2.2–12.4)	6.9 (3.7–16.1)	0.8676
Monocytes ($x10^9$/l)	m	1.3^{Aa} (0.3–2.3)	1.6^{a} (0.6–3.6)	2.3^{Bb} (0.8–3.7)	0.0051
	s	1.7^{A} (0.3–2.3)	1.9^{A} (0.4–4.6)	3.1^{B} (0.7–3.9)	0.0043
Granulocytes ($x10^9$/l)	m	6.1^{A} (1.5–15.4)	6.9^{A} (3.7–15.7)	3.8^{B} (1.8–11.2)	0.0002
	s	10.7 (2.9–24.3)	9.6 (2.1–19.0)	11.2 (4.1–23.2)	0.4891

Values in rows followed by different letters are significantly different: small letters at $P \leq 0.05$; capital letters at $P \leq 0.01$

in levels of white blood cell parameters in 21-day old suckling piglets and a slightly lower variation in 35-day old piglets.

It was recorded that younger piglets with an elevated CRP level, apart from the greater monocyte count, differed from the other piglets in terms of their reduced granulocyte count. Egeli et al. [7] and Svoboda et al. [23] reported lower neutrophil counts and their reduced percentage in comparison to healthy piglets assayed in blood of piglets (21- and 35-day old) with anemia caused by iron deficiency. An insufficient iron intake during the

period of intensive growth and development also disturbs erythropoiesis [7, 23]. Disturbance of homeostasis in iron metabolism also accompanies inflammations. In contrast, hypoferremia has a different underlying cause. It is triggered by the stimulation, e.g. by CRP, of the synthesis of proinflammatory cytokines, including IL-6 inducing hepcidin synthesis [2, 17]. An increase in its level inhibits iron absorption by enterocytes and its release by macrophages and hepatocytes, resulting in hypoferremia [1, 17]. Data in Table 2, referring to the red blood cell system, indicates that the younger offspring of dams with high blood CRP levels had lower hemoglobin and erythrocyte contents and lower hematocrit levels than the other piglets. The value of medians for these parameters indicates anemia in these animals. In blood of the other piglets the value of medians for these parameters fell within the lower limit of the recommended value [21]. It may be assumed that the incidence of anemia in piglets with elevated CRP levels may have been partly determined by the positive feedback between CRP and IL-6 [24]. Increased CRP concentration induces IL-6 and enhances its proinflammatory effect [9, 27]. IL-6 enhances synthesis of CRP [24] and hepcidin [17]. In blood of younger piglets with elevated CRP concentration the levels of MCV, MCH and RDW were higher than in the other piglets. Koorts et al. [11] reported that when inflammation is not accompanied by an increase in CRP concentration, the values of MCV, MCH and MCHC are within the recommended limits. When during inflammation the CRP level is elevated, also the MCV, MCH and MCHC levels increase above the limits. Although values of these indices (MCH and MCV) in piglets were within the limits of the physiological norm, the differences observed between piglets may have resulted from the varied CRP levels (Table 2). Such a possibility also shows high variability in erythrocyte volume in blood of piglets with elevated CRP concentration. Lippi et al. [14] indicated a positive correlation between RDW and CRP levels, recommending these indices in forecasting the incidence of atherosclerosis.

In turn, in older piglets the values of medians for the red blood cell parameters were within the physiological limits. However, it was stated that offspring of dams with a medium CRP level had lower hemoglobin contents, lower hematocrit and MCH levels in comparison to the values recorded in blood of offspring produced by dams with low CRP levels. In contrast, those piglets did not differ in this respect from offspring of dams with high CRP levels, except for hematocrit. It is difficult to interpret the cause for the incidence of anemia in younger piglets with elevated CRP levels. In the human we distinguish iron deficiency anemia (IDA) and immune response related anemia, i.e. anemia of chronic disease (ACD). It was shown that during ACD or IDA/ACD the concentrations of inflammatory markers (including CRP) increase in blood. In contrast, their level does not increase during IDA [4].

It is also assumed that the negative effect of elevated CRP levels on the values of the red blood cell parameters may have been caused by its role in the disturbance of antioxidant mechanisms [20]. Intensity of free radical generation, resulting from intensive metabolism, is observed in piglets between day 11 and 26 of life [28]. At the age of approx. 30 days uncontrolled oxidation processes take place in erythrocytes [19]. Sensitivity of erythrocytes to reactive oxygen species results in their destruction and as a consequence leads to anemia [10].

Table 2

The values of red blood cell and platelet indices in younger (m) and older (s) piglets with consideration of their origin

Indices	Age of piglets	Offspring of mothers with CRP levels:			P value
		low	medium	high	
Erythrocytes (x10^{12}/l)	m	5.7^{A} (2.8–7.6)	5.8^{A} (3.6–6.9)	3.8^{B} (2.3–4.6)	0.0001
	s	6.6 (4.9–10.0)	6.3 (0.7–7.3)	6,5 (5,4–7,9)	0.0726
Haemoglobin (g/l)	m	95^{A} (48–152)	96^{A} (57–134)	71^{B} (39–87)	<0.0001
	s	115^{a} (82–141)	106^{b} (12–131)	108^{ab} (81–137)	0.0007
MCV (fl)	m	50.8^{A} (38.9–63.4)	$50,1^{A}$ (40.2–62.0)	55.2^{B} (45.4–64.6)	0.0058
	s	51.0 (42.2–59.1)	48.5 (40.9–58.1)	50.8 (41.0–59.7)	0.0653
Ht (%)	m	28.4^{A} (12.5–43.3)	$30,0^{A}$ (16.7–38.6)	$20,6^{B}$ (13.4–28.0)	0.0002
	s	33.4^{a} (20.6–41.8)	31.3^{b} (13.0–40.5)	33.6^{a} (22.4–39.7)	0.0337
MCH (pg)	m	17.1^{A} (14.0–20.9)	16.8^{A} (13.6–19.9)	18.7^{B} (16.3–21.8)	0.0008
	s	17.3^{a} (15.5–24.1)	16.8^{b} (15.0–19.3)	16.7^{b} (15.0–19.4)	0.0024
MCHC (g/l)	m	338 (294–396)	334 (282–427)	331 (291–386)	0.1113
	s	345 (297–480)	342 (314–400)	340 (288–405)	0.4123
RDW (%)	m	16.9^{A} (11.0–27.2)	$16,9^{A}$ (11.8–25.5)	23.3^{B} (17.5–27.8)	0.0004
	s	16.5 (10.6–22.1)	17.0 (11.3–21.8)	15.7 (11.0–20.8)	0.3979
Platelets (x10^{9}/l)	m	895 (55–1837)	900 (346–1670)	1194 (533–2286)	0.1034
	s	906^{AB} (52–1744)	869^{A} (109–1597)	1179^{B} (55–1667)	0.0041
MPV (fl)	m	11.2^{A} (9.9–12.4)	$10,9^{B}$ (9.7–12.2)	10.9^{B} (10.3–13.3)	0.0005
	s	10.9 (10.0–13.3)	10.8 (9.9–11.9)	11.2 (1.3–12.6)	0.2272
LPTL (%)	m	0.99 (0.06–1.95)	1.09 (0.37–1.78)	1.25 (0.64–2.40)	0.1017
	s	1.0^{A} (0.05–1.45)	0.99^{A} (0.11–1.80)	1.29^{B} (0.06–2.99)	0.0051
PDW (%)	m	7.9^{A} (5.7–2.2)	7.2^{B} (5.5–9.1)	8.1^{A} (6.6–9.5)	0.0007
	s	7.3 (5.2–9.0)	7.2 (5.7–9.2)	7.9 (5.7–9.6)	0.0937

Values in rows followed by different letters are significantly different: small letters at P≤0.05; capital letters at P≤0.01

It was found that platelet counts in blood of piglets with elevated CRP levels considerably exceeded the physiological norm, ranging from 110 to 920 x 10^9/l [21]. Older piglets differed from the other offspring by greater platelet counts and higher percentage of large platelets (LPTL), indicating the induction of thrombocytopoiesis [12]. This condition may be caused both by iron deficit and by the action of proinflammatory mediators, primarily IL-6, being a stimulator of thrombopoietin [26].

It may be concluded based on the recorded results that low and medium serum CRP levels in blood of sows do not distinctly differentiate the concentration of this protein in their offspring. In turn, in piglets coming from dams with high CRP levels its level is higher than in the case of the other piglets. An elevated CRP concentration, irrespective of the age of piglets, was connected with an increased monocyte count, while in younger piglets it was associated with a reduced granulocyte count in blood. Moreover, it had an adverse effect on erythropoiesis in younger piglets, while in older piglets it was on thrombocytopoiesis.

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